TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		Attorney's Docket Number 045636-5053 U.S. Application No. Unassigned 10/048209
International Application. No.	International Filing Date	Priority Date Claimed
PCT/FR00/02174	July 28, 2000	July 30, 1999

Title of Invention: APPLICATIONS OF PEPTIDES DERIVED FROM THE CYTOPLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN (APP)

Applicants For EO/EO/US: Bernadette ALLINQUANT and Alain PROCHIANTZ

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. § 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9. ☐ An oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).
10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).

Items 11. to 14. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. § 3.28 and § 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☒ Other items or information:
    a. WO 01/09170
    b. PCT/IB/304
    c. PCT/IB/308
    d. International Search Report
    e. Statement Accompanying Sequence Listing
    f. Diskette containing Sequence Listing CRF
    g. Paper Copy of Sequence Listing
    h. PCT/IB/409 (in French)

JC13 Rec'd PCT/PTO 30 JAN 2002

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NUMBER
10/048209	PCT/FR00/02174	045636-5053
Unassigned		

15. ☒ The following fees are submitted:

Basic National Fee (37 C.F.R. § 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO.....$890.00
International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482).....$710.00
No international preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) but international search fee paid to USPTO (37 C.F.R. § 1.445(a)(2)).....$740.00
Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO.....$1,040.00
International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).	$

Claims	Number Filed	Number Extra	Rate	
Total Claims	9 - 20 =	0	X $18.00	$
Independent Claims	4 - 3 =	1	X $84.00	$ 84.00
Multiple dependent claim(s) (if applicable)			+ $280.00	$
		TOTAL OF ABOVE CALCULATIONS		$
		Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 C.F.R. §§ 1.9, 1.27, 1.28)		-$
		SUBTOTAL =		$
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)).				+$
		TOTAL NATIONAL FEE =		$890.00
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The Assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.230, 3.31). $40.00 per property				$
		TOTAL FEES ENCLOSED =		$
		Amount to be refunded		$
		Amount to be charged		$

a. ☒ A check in the amount of $890.00 to cover the above fees is enclosed.
b. ☒ Please charge my Deposit Account No. 50-0310 in the amount of **$84.00**
c. ☒ **Except** for issue fees payable under 37 C.F.R. § 1.130, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and § 1.17 which may be required, or credit any overpayment to Deposit Account No. 50-0310.

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Submitted: January 30, 2002

PATENT
ATTORNEY DOCKET NO. 45636-5053-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **Bernadette ALLINQUANT *et al.***
Application No.: (based on PCT/FR00/02174)
Filed: **January 30, 2002**
Group Art Unit: **Not Assigned**
Examiner: **Not Assigned**

For: **Applications of Peptides Derived From the Cytoplasmic Domain of Amyloid Precursor Protein (APP)**

BOX SEQUENCE
Commissioner for Patents
Washington, D.C. 20231

STATEMENT ACCOMPANYING SEQUENCE LISTING

Dear Sir:

The undersigned hereby states upon information and belief that the Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted concurrently herewith is identical to the written Sequence Listing submitted herewith.

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

Dated: January 30, 2002

By: Rachel B. Kapust
Rachel B. Kapust

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SEQUENCE LISTING

<110> CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE -CNRS
ALLINQUANT, Bernadette
PROCHIANTZ, Alain

<120> APPLICATIONS OF PEPTIDES DERIVED FROM THE CYTOPLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN (APP)

<130> 45636-5053-US

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PATENT
Attorney Docket No. 045636-5054

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bernadette ALLINQUAT, et al.	
	Group Art Unit: Unassigned
U.S. National Phase Application Filed: January 30, 2002	Examiner: Unassigned
U.S. Application No.: To Be Assigned	
Date of National Stage Entry : Concurrently	
Based on PCT/FR00/02174 Filed : July 28, 2000	
For: NOVEL APPLICATIONS OF PEPTIDES DERIVED FROM THE CYTOPLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN	

Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above-identified application on the merits, please amend the application as follows:

IN THE SPECIFICATION:

Please substitute the following paragraph for the paragraph bridging pages 1 and 2.

Alzheimer's disease is a neurodegenerative disorder which affects from 1 to 6% of the population over the age of 65. One of its characteristics is the presence of senile plaques which contain β-amyloid (βA4 or BAP), which is a toxic product derived from APP and consisting of peptides of 39 to 42 amino acids, which are engendered by cleavage of APP by two proteases, β- and γ-secretase. Moreover, a third enzyme, named α-secretase, cleaves APP between the β- and γ-sites, therefore making it impossible to form the supposedly pathogenic βA4. None of these secretases has, to date, been identified, even though there are legitimate suspicions regarding the PS1 protein (product of the Presenilin-1 gene, mutated in familial forms of Alzheimer's disease). In fact, PS1 may be either γ-secretase or one of its cofactors. Finally, other cleavage sites exist in the C-terminal domain, including the site for caspases (N. Barnes et al., J. Neuroscience, 1998, 18, 15, 5869-5880), between the aspartate and alanine residues of SEQ ID NO: 1 (positions 16 and 17). It remains that the mechanisms responsible for the toxicity of βA4 are unknown and that the relationship between the presence of βA4 in the plaques and the pathological condition has not been elucidated. It is probable that other factors and/or other domains of the molecule are also involved.

IN THE CLAIMS:

Please cancel claims 1-11 and add the following claims 12-20.

12. A peptide selected from the group of consisting of $Y_1KQYTSIHHGY_0$ (SEQ ID NO: 2), $Y_1KKQYTSIHHGY_0$ (SEQ ID NO: 3) and $Y_1KKKQYTSIHHGY_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 represents an internalization and addressing peptide corresponding to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16,}$ in which $X_{1,}X_{2,}X_{3,}X_{4,}X_{5,}X_{6,}X_{7,}X_{8,}X_{9,}X_{10,}X_{11,}X_{12,}X_{13,}X_{14,}X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan.

13. The peptide as claimed in claim 12, wherein the sequence Y_1 corresponds to the sequence KQIKIWFQNRRMKWKK (SEQ ID NO: 5).

14. A method of selecting and screening products capable of inhibiting apoptosis comprising detecting inhibition of the capacity of the juxtamembrane domain located between positions 649 and 664 of the cytoplasmic domain of amyloid precursor protein to induce apototic activity subsequent to internalization into a cell.

15. The method of claim 14, wherein said peptide is combined with an internalization peptide selected from the group consisting of internalization peptides capable of crossing the blood-brain barrier.

16. A method of selecting and screening products capable of inhibiting apoptosis comprising detecting inhibition of the capacity of a peptide selected from the group consisting of Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2), Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3) and Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null or represents an internalization and addressing peptide corresponding to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$, in which $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan, to induce apototic activity subsequent to internalization into a cell.

17. The method of claim 16 wherein candidate inhibitors are tested against cells in which the claimed peptide has been internalized.

18. The method of claim 17 comprising the steps of:

bringing the potential inhibitor into contact with said cell into which said peptide has been internalized, and

either measuring cleavage of DNA or of actin or measuring the p20 subunit of caspase 3.

20. A peptide selected from the group consisting of peptides Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2) and Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null, and of the peptide of formula Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 represents VVEVD and Y_1 is null.

19. A method of treating cancer comprising the administration of an effective amount of a peptide of claim 12.

20. A peptide selected from the group of peptides Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2) and Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null, and of the peptide of formula Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 represents VVEVD and Y_1 is null.

Attorney Docket No.: 045636-5054
Application No.: Unassigned

REMARKS

Applicants respectfully submit that no prohibited new matter has been introduced by this Preliminary Amendment and that amended claims 12-20 are drawn to the same invention as claims 1-11 of International Application PCT/FR00/00217. The changes to the claims represent changes in formalities so as to bring the claims into compliance with the rules of practice in the United States, by avoiding improper multiple dependencies and eliminating multiple dependencies to reduce costs; and to eliminate improper "use" claims.

Respectfully Submitted,

MORGAN, LEWIS & BOCKIUS LLP

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Date: January 30, 2002

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Attorney Docket No.: 045636-5054
Application No.: Unassigned

MARKED UP VERSION SHOWING CHANGES

As to the paragraph bridging pages 1 and 2, note change in residue positions referred to:

Alzheimer's disease is a neurodegenerative disorder which affects from 1 to 6% of the population over the age of 65. One of its characteristics is the presence of senile plaques which contain β-amyloid (βA4 or BAP), which is a toxic product derived from APP and consisting of peptides of 39 to 42 amino acids, which are engendered by cleavage of APP by two proteases, β- and γ-secretase. Moreover, a third enzyme, named α-secretase, cleaves APP between the β- and γ-sites, therefore making it impossible to form the supposedly pathogenic βA4. None of these secretases has, to date, been identified, even though there are legitimate suspicions regarding the PS1 protein (product of the Presenilin-1 gene, mutated in familial forms of Alzheimer's disease). In fact, PS1 may be either γ-secretase or one of its cofactors. Finally, other cleavage sites exist in the C-terminal domain, including the site for caspases (N. Barnes et al., J. Neuroscience, 1998, 18, 15, 5869-5880), between the aspartate and alanine residues of SEQ ID NO: 1 (positions ~~[15]~~ **16** and ~~[16)]~~ **17)**. It remains that the mechanisms responsible for the toxicity of βA4 are unknown and that the relationship between the presence of βA4 in the plaques and the pathological condition has not been elucidated. It is probable that other factors and/or other domains of the molecule are also involved.

APPLICATIONS OF PEPTIDES DERIVED FROM THE CYTOPLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN (APP)

The present invention relates to novel applications of peptides derived from the cytoplasmic domain of amyloid precursor protein (APP).

Amyloid precursor protein APP, is a protein of unknown function, the neuronal form of which comprises 695 amino acids; it has a single transmembrane domain (positions 625-648) and a short 47 amino acid cytoplasmic domain (positions 649-695) represented in the attached sequence listing under the number SEQ ID NO:1.

Alzheimer's disease is a neurodegenerative disorder which affects from 1 to 6% of the population over the age of 65. One of its characteristics is the presence of senile plaques which contain β-amyloid (βA4 or BAP), which is a toxic product derived from APP and consisting of peptides of 39 to 42 amino acids, which are engendered by cleavage of APP by two proteases, β- and γ-secretase. Moreover, a third enzyme, named α-secretase, cleaves APP between the β- and γ-sites, therefore making it impossible to form the supposedly pathogenic βA4. None of these secretases has, to date, been identified, even though there are legitimate suspicions regarding the PS1 protein (product of the Presenilin-1 gene, mutated in familial forms of Alzheimer's disease). In fact, PS1 may be either γ-secretase or one of its cofactors. Finally, other cleavage sites exist in the C-terminal domain, including the site for caspases (N. Barnes et al., J. Neuroscience, 1998, 18, 15, 5869-5880), between the aspartate and alanine residues of SEQ ID NO: 1 (positions 15 and 16). It remains that the mechanisms responsible for the toxicity of βA4 are unknown and that the relationship between the presence of βA4 in the plaques and the pathological condition has not been

elucidated. It is probable that other factors and/or other domains of the molecule are also involved.

For this reason, many studies have tried to establish the physiological and/or physiopathological role of APP and of the various products of its metabolism. In fact, the physiological ligand, if it exists, of the N-terminal domain has not been identified and the signalling pathways are still poorly defined. One of the strategies for making it possible to analyze these signalling pathways is the identification of molecular partners of the cytoplasmic domain.

The cytoplasmic domain of APP, and also various peptides derived from this cytoplasmic domain, have in particular been studied:

- the sequences YTSI, KKKQYTSIHHGVVEV (SEQ ID NO: 8), GYENPTY (SEQ ID NO: 9) and NPTY have been identified as internalization signals; more precisely, they are considered to be sequences for transcytosis of APP between the basolateral and apical compartments of MDCK epithelial cells (Haass et al., J. Cell Biol., 1995, 128, 4, 537-547; Lai et al., J. Biol. Chem., 1995, 270, 8, 3565-3573; Lai et al., J. Biol. Chem., 1998, 273, 6, 3732-3739);

- the C-terminal cytoplasmic domain (APP-Cter) has been identified as:

. being involved in regulating the GTPase activity of the αo subunit of heterotrimeric G protein (Brouillet et al., J. Neuroscience, 1999, 19, 5, 1717-1727);

. interacting with several proteins: Pat-1 interacts with the juxtamembrane domain (KKKQYTSIHHG) and with the complete C-terminal domain and is thought to be involved in transporting APP along microtubules, toward the cell surface (Zheng et al., PNAS, 1998, 95, 14745-

14750); the αo subunit of heterotrimeric G protein interacts with the median region of said C-terminal cytoplasmic domain, at the histidine doublet (HH) (Nishimoto et al., Nature, 1993, 362, 75-79) and the Fe65 protein with the most distal region of the APP-Cter domain (Fiore et al., J. Biol. Chem., 1995, 270, 52, 30853-30856).

These various results show the complexity of the mechanisms in which amyloid precursor protein (APP) is involved.

The inventors have now shown that, surprisingly, peptides comprising the juxtamembrane domain (positions 649-664) of the cytoplasmic domain of amyloid precursor protein (APP) have, after internalization into cells, apoptotic activity.

A subject of the present invention is peptides, charcterized in that they consist of sequences which include the juxtamembrane domain of the cytoplasmic domain of amyloid precursor protein (APP) (one-letter code), and which are selected from the group consisting of the sequences Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2), Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3) and Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 represents an internalization and addressing peptide derived from the 3rd helix of homeodomains, and from structurally related peptides, and preferably corresponds to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$, in which $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan.

Among the preferred Y_1 sequences, mention may be made of the sequence KQIKIWFQNRRMKWKK (SEQ ID NO: 5).

The peptides $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$ have in particular been described in International Application WO 97/12912.

The peptides according to the invention cause apoptosis of the cells into which they are internalized and may advantageously be used to select and screen products capable of inhibiting cellular apoptosis.

Another subject of the present invention is therefore also the use of a peptide comprising the juxtamembrane domain of the cytoplasmic domain of amyloid precursor protein (APP), for selecting and screening products capable of inhibiting apoptosis.

According to an advantageous embodiment of said use, the peptide comprising the juxtamembrane domain of the cytoplasmic domain of amyloid precursor protein (APP) is combined with an internalization peptide selected from the group consisting of peptides capable of crossing the blood-brain barrier.

By way of examples of internalization peptides which can be used in the present invention, mention may be made of:

- internalization and addressing peptides derived from the 3rd helix of homeodomains and peptides structurally related to the latter,

- peptides derived from viral proteins: VP22 (G. Elliott et al., Cell, 1997, 88, 223-233; A. Prochiantz, Current Opinion in Cell Biology, 2000, 12, 399-406); peptides derived from the HIV Tat protein transduction domain (Schwarze SR et al., Science, 1999, 285, 5433, 1569-1572),

- and also other peptides, such as those described in A. Prochiantz, 2000, mentioned above; M. Lindgren et

al., TIPS, 2000, 21, 99-103 or C. Rousselle et al., Mol. Pharmacol., 2000, 57, 679-686 (amphiphilic peptides, peptides derived from signal sequences, transportan, etc.).

Preferably, the peptide used in the present invention is selected from the group consisting of the sequences (one-letter code) $Y_1KQYTSIHHGY_0$ (SEQ ID NO: 2), $Y_1KKQYTSIHHGY_0$ (SEQ ID NO: 3) and $Y_1KKKQYTSIHHGY_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null or represents an internalization and addressing peptide derived from the 3rd helix of homeodomains, and from structurally related peptides, and preferably corresponds to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$, in which $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan.

The peptide of SEQ ID NO: 2 in which Y_1 is null and Y_0 is null is named peptide G (see also figure 1).

The peptide of SEQ ID No. 4 in which Y_1 is null and Y_0 represents VVEVD is named Jcasp (or Gcasp).

Another subject of the present invention is also the use of cells, into which a peptide as defined above has been internalized, for selecting and screening products capable of inhibiting apoptosis.

Another subject of the present invention is also a method for screening and selecting products capable of inhibiting apoptosis, characterized in that it comprises:

- bringing the potential inhibitor into contact with a cell into which a peptide as defined above has been internalized, and

- measuring cleavage of DNA (revealed in particular by TUNEL labeling) or of actin (revealed, for example, with an anti-fractin antibody) or measuring the p20 subunit of caspase 3 (for example by specific labeling).

Another subject of the present invention is also the use of a peptide as defined above, for preparing an anticancer medicinal product.

Another subject of the present invention is also peptides, characterized in that they are selected from the group consisting of the sequences (one-letter code) Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2) and Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null, and of the peptide of formula Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 represents VVEVD and Y_1 is null.

The invention will be more clearly understood by virtue of the attached figures in which:

- figure 1 represents the sequence of the cytoplasmic domain of amyloid precursor protein (APP): positions 649-695, and also some of its fragments: peptide G: positions 651-659; peptide E: positions 663-671; peptide H; positions 680-688; peptide Jcasp: positions 649-664,

- figures 2 and 3 represent the quantification of the cleavage of DNA using the TUNEL technique, 24 h after internalization of the peptides,

- figures 4 and 5 represent the quantification of the cleavage of actin by caspase 3, using an anti-fractin antibody,

- figure 6 illustrates detection of p20 in a neuron by immunolabeling (arrow) (alkaline phosphatase); the p20 is present in all compartments; scale: 10 μm,

- figure 7 illustrates activation of p20 by peptide Jcasp (2.4 μM),

- figure 8 illustrates the results obtained *in vivo*: representative diagrams (one experiment, one animal per condition) of the distribution of fractin-positive cells in adjacent sections. The value 0 is arbitrarily attributed to the site of injection. The peptide Jcasp shows a greater number of fractin-positive cells compared to peptide J(Y→D)casp or to the control.

EXAMPLE 1: Materials and Methods

1.1 Primary cultures of neurons

Cortical and corticostriatal neurons are prepared, as described previously (Lafont et al., Development, 1992, 114, 17-29), from E14 mouse embryos or from E15 rat embryos.

Briefly, the dissociated cells are plated out onto polyornithine-coated plastic plates (ELISA-type plates) at a density of 5,000 cells per well, and incubated in a suitable medium supplemented with hormones, proteins and salts.

In order to verify the internalization of the peptide studied, the cells are plated out onto polyornithine-coated glass slides at a density of 100,000 cells per slide.

1.2 Preparation of peptides

The V1 vector (Penetratin or P = KQIKIWFQNRRMKWKK) (SEQ ID NO: 5) is used as an internalization peptide which,

after genetic or chemical fusion to a cargo, allows the translocation thereof across the plasma membrane and the cytoplasmic and nuclear addressing thereof.

Several peptides were thus prepared:

. SEQ ID NO: 5 + entire cytoplasmic domain of APP (SEQ ID NO: 1).

. Y_1KK**KQYTSIHHG**Y_0: SEQ ID NO: 4 in which Y_0 is null or represents VVEVD (Jcasp) and Y_1 represents SEQ ID NO: 5; the portion in bold corresponds to peptide G of figure 1.

. Y_1**KQYTSIHHG**Y_0: SEQ ID NO: 2 in which Y_0 is null (peptide G) and Y_1 represents SEQ ID NO: 5; the portion in bold corresponds to peptide G of figure 1.

. Y_1K**KQYTSIHHG**Y_0; SEQ ID No. 3 in which Y_0 is null and Y_1 represents SEQ ID No. 5; the portion in bold corresponds to peptide G of figure 1.

. SEQ ID NO: 5 + domain E (VDAAVTPEE, SEQ ID NO: 6), underlined in the sequence according to figure 1.

. SEQ ID NO: 5 + domain H (NGYENPTYK, SEQ ID NO: 7), underlined in the sequence according to figure 1.

. SEQ ID NO: 5 + peptide corresponding to the MYC sequence [EQKLISEED] (Pmyc peptide).

. SEQ ID NO: 5 + peptide J(Y→D)casp.

Peptide G corresponds to a transcytosis signal and comprises a tyrosine residue (Y); the peptide was also internalized either after phosphorylation of this tyrosine (Y-P) or after its substitution with an alanine (Y→A) or an aspartate (Y→D). The two substitutions totally abolish the physiological effects

of G, whereas phosphorylation reduces them without abolishing them. Insofar as Y→D mimics a phosphorylation, it may be proposed, as a parcimonious hypothesis, that the tyrosine is necessary, but that the phosphorylation thereof probably is not, the intermediate effect of Y-P possibly then being explained by dephosphorylation of the peptide in the cell. It cannot, however, be excluded that phosphorylation is necessary but that the substitution Y→D is not sufficient to mimic it.

These various peptides are synthesized chemically (95-98% purity, Synthem, France) with (Jcasp and J(Y→D)casp) or without N-terminal biotin and an aminopentanoic acid spacer arm (Derossi et al., J. Biol. Chem., 1994, 269, 10444-10450).

It should be noted that, since the last 2 amino acids of the sequence SEQ ID NO: 5 are lysines (KK), peptide G (KQYTSIHHG) is artificially extended by 2 amino acids.

1.3 Internalization of the recombinant peptides into neurons

The internalization conditions are the same as those described in International Application WO 97/12912.

All the peptides are added to the cells two hours after the latter have been plated out. The internalization is verified by confocal microscopy after immunolabeling (Pmyc) or detection of biotin (Jcasp and its variants).

The internalization and the intracellular stability of Jcasp, Pmyc and J(Y→D)Casp are identical. The irreversible caspase inhibitors zVAD-fmk (100 µM) and zDEVD-fmk (200 µM) (Calbiochem, France) are added 1 hour before addition of the peptide.

1.4 Immunocytochemistry and quantification of apoptotic cells

The apoptotic cells are detected by TUNEL labeling (fluorescein or alkaline phosphatase kits) as described by the supplier (Roche Diagnostics, France).

For the immunodetection of the fractin or of the p20 subunit of caspase 3 (Pharmingen), the cells are fixed with 4% paraformaldehyde (30 minutes, room temperature), washed three times with PBS and saturated for 1 hour at 37°C with 10% fetal calf serum (FCS) in PBS containing 0.2% of Triton X 100.

Purified primary antibodies directed against fractin or p20 are diluted (in PBS-FCS) 2000-fold and 500-fold, respectively, incubated overnight at 4°C washed three times and incubated with biotinylated anti-rabbit antibodies.

The detection is carried out using the alkaline phosphatase amplification kit (Vector, France).

For each condition, 600 to 800 cells are counted three times.

The statistical analysis is carried out with ANOVA and the Scheffé test.

1.5 *In vivo* tests

1 µl (0.2 µl/min) of 2.7 µM of Jcasp (n = 8) or J(Y→D)Casp (n = 6), or of PBS (n = 3) is injected stereotactically into the cortex of adult mice with the co-ordinates A=0, L=2 and D=1.5 (mouse brain Atlas by KBJ Franklin and G. Paxinos, *Academic Press*). 24 hours later, the animals are sacrificed and perfused with 4% paraformaldehyde, and the brains are extracted and cryoprotected.

Frozen sections (16 µm thick) are prepared and used for TUNEL detection or detection by fractin immunocytochemistry, using the purified primary antibody (1/100th dilution in PBS-FCS) without amplification and an anti-rabbit secondary antibody labeled with Cy3 and diluted to 1/400th (Jackson Immunoresearch Laboratories, Inc.). The number of fractin-positive cells is counted on adjacent sections. The statistical analysis is carried out by ANOVA and the Fischer test.

EXAMPLE 2: ***In vitro* results**

2.1. Induction of neuronal apoptosis

Internalization of the entire C-ter domain (APP-Cter) is not toxic but has, however, a negative effect on neurite growth. The internalization of peptides E and H has no effect, whereas that of peptide G, at concentrations lower than one µM, reproduces the effects of the intact C-terminal domain.

The most advantageous result is that peptide G, at concentrations of the order of 1 to 1.5 µM, or peptide Jcasp, at concentrations of 1.2 to 2.4 µM, causes neuronal death, and that this death corresponds to an apoptotic, and therefore regulated, process.

The apoptotic nature of the death caused by the internalization of peptide G or of peptide Jcasp (figures 2-7) is demonstrated by the DNA fragmentation, revealed by the "TUNEL" method (figures 2 and 3), and by the activation of caspases (figures 4-7). The activation of caspases is demonstrated by the appearance of cleaved forms of actin and by the blocking of apoptosis by caspase inhibitors with a broad spectrum of activity (inhibitor of caspase 1, 3,

4 and 7), such as zVAD or zDEVD-fmk (figures 4, 5 and 7), more specific for caspase 3.

Figures 2 and 3 illustrate the quantification of the DNA cleavage by the TUNEL technique 24 h after internalization of the peptides.

Peptide G was internalized at 2 concentrations (1X and 2X) and peptide Gcasp (or Jcasp) was internalized at the 1X concentration, in the presence or absence of the caspase inhibitor zVAD.

Each condition was tested in triplicate. The percentage of positive cells was evaluated after 24 h by counting approximately 1000 cells per well. The graph indicates a significant increase in the DNA cleavage in the presence of peptide G alone (concentration 1X: $p<0.0001$; concentration 2X: $p<0.0001$) and of peptide Jcasp (or Gcasp)(KKKQYTSIHHGVVEVD) (SEQ ID NO. 4 in which Y_0 = VVEVD and Y_1 = SEQ ID NO: 5) (concentration 1X:$p<0.0001$). The ZVAD inhibits this increase in cleavage.

Peptide Jcasp induces neuronal apoptosis. Two hours after plating out onto plates, peptide Jcasp is added to the E15 rat cortical neurones and cell death is evaluated by the TUNEL effect, 24 hours later. Figure 3 shows that peptide Jcasp (1.2 and 2.4 µM) produces DNA fragmentation.

Substitution of the tyrosine with an aspartate decreases cell death, as for peptide G, whereas the internalization of a myc peptide which has no relation to APP and is linked to penetratin (Pmyc) has no effect on the number of positive cells obtained by the TUNEL method.

Since the DNA fragmentation suggests apoptosis, the same experiment was carried out in the presence of

zDEVD-fmk, which inhibits caspase 3. At 200 µM, zDEVD-fmk has a weak effect on basal cell death and inhibits the DNA fragmentation induced by peptide Jcasp at 1.2 µM and 2.4 µM (figure 3).

Similar inhibitions are obtained with the inhibitor zVAD-fmk (100 µM), (see figure 2).

Peptide Jcasp not linked to penetratin (internalization sequence of SEQ ID NO: 5), which is therefore not internalized, does not induce any DNA fragmentation.

2.2. <u>The induction of apoptosis is linked to the activation of caspase 3</u>

- Quantification of the actin cleavage by caspase 3 using an anti-fractin antibody.

Figures 4 and 5 illustrate the quantification of the actin cleavage by caspase 3 using the anti-fractin antibody, by immunocytochemistry after fixing the cells with paraformaldehyde (F. Yang et al., Am. J. Pathol., 1998, 152, 2, 379-389). The anti-fractin antibody specifically recognizes actin cleaved by caspase 3. The percentage of fractin-positive neurons was determined after 24 h of internalization of the peptides, by counting approximately 1000 cells per well in triplicate. In the presence of peptides G (KQYTSIHHG = SEQ ID NO: 2 in which Y_1 represents an internalization and addressing peptide, as defined above, and Y_0 is null) (1X and 2X) and Gcasp (or Jcasp) (KKKQYTSIHHGVVEVD = SEQ ID NO: 4 in which Y_1 represents an internalization and addressing peptide, as defined above, and Y_0 represents VVEVD) (1X), there is a significant increase in the cleavage by actin (G1X: $p<0.0003$; G2X: $p<0.0001$; Gcasp1X: $p<0.0001$). The zVAD alone inhibits all endogenous cleavage, by caspases, of neurones and significantly inhibits the increase in this cleavage with G1X, G2X and Gcasp1X (G1X/zVADG1X: $p<0.0001$;

G2X/zVADG2X: $p<0.0001$; Gcasp/zVADGcasp: $p<0.0001$), even though the inhibition is not complete.

Figure 5 shows that peptide Jcasp induces actin cleavage. It also shows that peptide J(Y→D)casp is relatively inactive and that the inhibitor zDEVD-fmk, which is more specific for caspase 3, inhibits the actin cleavage induced by peptide Jcasp.

- quantification of the actin cleavage by caspase 3 by measuring p20

In order to verify that caspase 3 is effectively involved in the apoptosis caused by peptide Jcasp, use is made of the fact that this enzyme (caspase 3) is synthesized in the form of a propetide (37 kDa) which, after stimulation, generates an active subunit of 17-22 kDa (p20).

Immunoreactivity for p20 is examined in mouse cortical embryonic neurons cultured for 24 hours in the presence of several peptides. Figure 6 illustrates the immunoreactivity for the p20 protein and figure 7 quantifies the induction of the p20.

Peptide Jcasp (2.4 µM) induces maturation of p20; significantly less effect is obtained with peptide J(Y→D)casp, confirming the importance of the tyrosine residue in caspase 3 induction.

The inhibitor zDEVD-fmk significantly decreases the activation of p20, suggesting that the apoptosis induced by peptide Jcasp involves maturation of caspase 3.

The inventors have therefore clearly shown the pro-apoptotic nature of peptide G internalized by virtue of its linkage to vector V1.

Such a property is of interest for the following reasons:

1. The entire C-terminal domain is not pro-apoptotic.

2. There is a cleavage site for caspases between the aspartate (D) and alanine (A) residues marked in bold in the sequence of APP-Cter (figure 1).

It is therefore possible to put forward the hypothesis that the cleavage between D and A unmasks a sequence KKKQYTSIHHGVVEVD (= SEQ ID NO: 4, in which Y_1 is null and Y_0 represents VVEVD) with apoptotic activity. This is particularly important since it reveals a mechanism involved in the neuronal loss which accompanies dementias of the Alzheimer type.

The first advantage of having identified an APP-derived peptide corresponding to a domain, normally exposed after *in vivo* cleavage and capable of causing cells to enter into apoptosis is to propose an original mechanism which may shed light on certain aspects of Alzheimer pathology and therefore to discover novel therapeutic approaches (development of inhibitors).

Moreover, the linking of the G sequence to the V1 vector makes it possible to produce a peptide which, once internalized by neurons in culture, brings about their apoptosis. Because of the properties of V1, entry occurs in 100% of the cells, whatever their degree of maturation *in vitro*, and these cells are normal (primary cultures).

The aspartate at position 664 corresponds to the cleavage site for caspase; the peptide according to the invention, when it is internalized into neuronal cells, activates caspase 3, causes actin cleavage at a caspase-sensitive site and induces DNA fragmentation.

Surprisingly, the peptides in accordance with the invention which do not comprise BAP, thus have pro-apoptotic properties both *in vitro* and *in vivo*.

EXAMPLE 3: ***In vivo* results**

The *in vitro* results (see example 2) demonstrate that the internalization of Jcasp by mouse or rat cortical neurons causes apoptosis by activation of a caspase and suggest that caspase 3 is one of the caspases activated.

In order to verify whether peptide Jcasp is also active *in vivo*, and in the adult brain, 1 µl of peptide Jcasp, of peptide J(Y→D)casp (each at 2.7 µM in a saline buffer) or of saline buffer is injected into the mouse cerebral cortex. 24 hours later, the number of fractin-positive cells is quantified on each side of the injection site.

Figure 8 illustrates the results obtained. Although variations were observed between the various experiments, they all gave similar results showing a considerable and specific effect of peptide Jcasp on the number of fractin-positive cells (mean ± SEM: Jcasp (8 animals), 40.7 ± 10.9; J(Y→D)casp (6 animals), 13 ± 3.2; control (3 animals): 8 ± 8; Jcasp vs control: $p<0.05$; Jcasp vs J(Y→D)casp: $p<0.04$; control vs J(Y→D)casp:NS).

In vivo applications, with infusion of the peptide using mini pumps, are also possible.

On this basis, this system constitutes a rapid and simple test for screening libraries of products which act specifically on the apoptotic death induced by this peptide, and are inoffensive on other models of apoptosis.

The identification of such substances is therefore very useful for developing treatments for the apoptosis which accompanies Alzheimer's disease.

CLAIMS

1. A peptide, characterized in that it consists of a sequence which includes the juxtamembrane domain of the cytoplasmic domain of amyloid precursor protein (βAPP) (one-letter code), and which are selected from the group consisting of the sequences Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2), Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3) and Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 represents an internalization and addressing peptide derived from the 3rd helix of homeodomains, and from structurally related peptides.

2. The peptide as claimed in claim 1, characterized in that said internalization and addressing peptide corresponds to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$, in which $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan.

3. The peptide as claimed in claim 1 or claim 2, characterized in that the sequence Y_1 corresponds to the sequence KQIKIWFQNRRMKWKK (SEQ ID NO: 5).

4. The use of a peptide comprising the juxtamembrane domain of the cytoplasmic domain of amyloid precursor protein (APP), for selecting and screening products capable of inhibiting apoptosis.

5. The use as claimed in claim 4, characterized in that said peptide is combined with an internalization peptide selected from the group consisting of internalization peptides capable of crossing the blood-brain barrier.

6. The use as claimed in either of claims 4 and 5, characterized in that said peptide is selected from the group consisting of the sequences (one-letter code) $Y_1KQYTSIHHGY_0$ (SEQ ID NO: 2), $Y_1KKQYTSIHHGY_0$ (SEQ ID NO: 3) and $Y_1KKKQYTSIHHGY_0$ (SEQ ID NO: 4), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null or represents an internalization and addressing peptide derived from the 3rd helix of homeodomains, and from structurally related peptides.

7. The use as claimed in any one of claims 4 to 6, characterized in that said internalization peptide corresponds to the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}$, in which $X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}$ and X_{16} each represent an α-amino acid, 6 to 10 of said amino acids being hydrophobic and X_6 representing a tryptophan.

8. The use of cells, into which a peptide as defined in claims 4 to 7 has been internalized, for selecting and screening products capable of inhibiting apoptosis.

9. A method for screening and selecting products capable of inhibiting apoptosis, characterized in that it comprises:

- bringing the potential inhibitor into contact with a cell into which a peptide as defined in claims 4 to 7 has been internalized, and

- measuring cleavage of DNA or of actin or measuring the p20 subunit of caspase 3.

10. The use of a peptide as defined in claims 4 to 7, for preparing an anticancer medicinal product.

11. A peptide, characterized in that it is selected from the group consisting of the sequences (one-letter code) Y_1KQYTSIHHGY$_0$ (SEQ ID NO: 2) and Y_1KKQYTSIHHGY$_0$ (SEQ ID NO: 3), in which Y_0 is null or represents V, VV, VVE VVEV or VVEVD and Y_1 is null, and of the peptide of formula Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO: 4), in which Y_0 represents VVEVD and Y_1 is null.

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En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

(54) Title: USES OF PEPTIDES DERIVED FROM THE CYTOPLASMIC DOMAIN OF THE AMYLOID PROTEIN PRECURSOR (APP)

(54) Titre: APPLICATIONS DE PEPTIDES ISSUS DU DOMAINE CYTOPLASMIQUE DU PRECURSEUR DE LA PROTEINE AMYLOIDE (APP)

(57) Abstract: The invention concerns novel uses of peptides derived from the cytoplasmic domain of the amyloid protein precursor (APP); said peptides are in particular sequences including the membrane domain juxtaposed to the cytoplasmic domain of the amyloid protein precursor (APP) (one-letter code), selected in the group consisting of the sequences Y_1KQYTSIHHGY$_0$ (SEQ ID NO:2), Y_1KKQYTSIHHGY$_0$ (SEQ ID NO:3) and Y_1KKKQYTSIHHGY$_0$ (SEQ ID NO:4), wherein Y_0 is nil or represents V, VV, VVE, VVEV or VVVED and Y_1 represents an internalisation and addressing peptide, derived from the 3rd helix of homeodomains and structurally related peptides. The invention also concerns the use of a peptide comprising the membrane domain juxtaposed to the cytoplasmic domain of the amyloid protein precursor (APP), for selecting and screening products capable of inhibiting apoptosis.

(57) Abrégé: Nouvelles applications de peptides issus du domaine cytoplasmique du précurseur de la protéine amyloïde (APP); lesdits peptides sont notamment constitués par des séquences incluant le domaine juxtamembranaire du domaine cytoplasmique du précurseur de la protéine amyloïde (APP) (code une lettre), sélectionnées dans le groupe constitué par les séquences Y_1KQYTSIHHGY$_0$ (SEQ ID NO :2), Y_1KKQYTSIHHGY$_0$ (SEQ ID NO :3) et Y1KKKQYTSIHHGY$_0$ (SEQ ID NO :4), dans lesquelles Y_0 est nul ou représente V, VV, VVE VVEV ou VVEVD et Y_1 représente un peptide d'internalisation et d'adressage, issu de la 3ème hélice des homéodomaines et de peptides structurellement apparentés. Utilisation d'un peptide comprenant le domaine juxtamembranaire du domaine cytoplasmique du précurseur de la protéine amyloïde (APP), pour la sélection et le criblage de produits aptes à inhiber l'apoptose.

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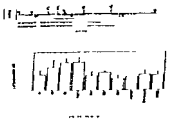

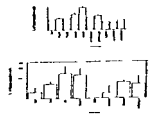

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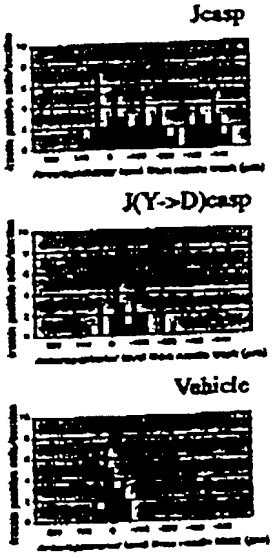

FIGURE 8

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As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

APPLICATIONS OF PEPTIDES DERIVED FROM THE CYTOPOLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN (APP)

the specification of which:

is attached hereto; or

was filed as United States application Serial No. 10/048,209 on January 30, 2002 and was amended on _____(if applicable); or

was filed as PCT international application Number PCT/FR00/02174 on July 28, 2000 and was amended under PCT Article 19 on _____(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office information which is material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN APPLICATION(S):

COUNTRY (if PCT, indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
France	99/09929	30 July 1999	[X] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No

Combined Declaration For Patent Application and Power of Attorney - **(Continued)**
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO.: 045636-5053

I hereby claim the benefits under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

U.S. PROVISIONAL APPLICATIONS

U.S. PROVISIONAL APPLICATION NO.	U.S. FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or §365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT:

U.S. APPLICATIONS		STATUS (Check One)		
U.S. APPLICATION NO.	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the registered practitioners of Morgan, Lewis & Bockius LLP included in the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number.

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Direct Telephone Calls To:
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202-739-3000

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Listing of Inventors Continued on attached page(s) [ ] Yes [X] No

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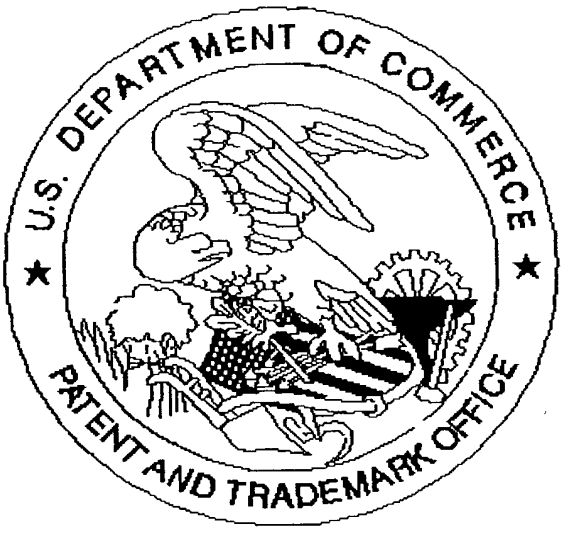

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